

## ABSTRACT

The invention described herein comprises methods for stabilizing polypeptides and polypeptide complexes, and the polypeptides and polypeptide complexes stabilized using the methods. To achieve stabilization, a cross-link reaction is controlled such that polypeptides and polypeptide complexes maintain their original functionality. In one embodiment, the invention provides a method for the identification of amino acid residues which, when cross-linked, are least disruptive to the structure and function of the polypeptide or polypeptide complex. In another embodiment, the invention provides a method for mutagenesis of identified residues to further control the cross-link reaction.

Polypeptides and polypeptide complexes so stabilized can be utilized under a wide variety of physiological and non-physiological conditions. Further, the cross-link methodology disclosed herein may preclude the need for addition of exogenous structures to engineered proteins and complexes, such as peptide linkers that could be immunogenic and/or significantly decrease efficacy. In another embodiment, the invention provides a method for statistical analysis of databases of structural and/or sequence information available for polypeptides and polypeptide complexes to be stabilized. The statistical analysis identifies suitable residue pairs which are least likely to be disruptive of structure and function when cross-linked. Further, in a polypeptide chain or chains to be cross-linked, potentially undesirable reactive side-chains may be masked and protected, or altered using site-directed mutagenesis, *e.g.*, to introduce a maximally conservative point mutation that will not support the cross-link reaction. The cross-link reaction conditions may also be adjusted to prevent undesired cross-links or other undesired side-effects. At residues identified as desirable positions for cross-linking, reactive side-chains may be introduced by site-directed mutagenesis, and the cross-link reaction is carried out using the conditions identified above.